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Mitochondria with loosely and tightly coupled oxidative phosphorylation in skeletal muscle

From skeletal muscle of humans, suffering from muscle disease, mitochondria can be isolated which sometimes show loosely coupled oxidative phosphorylation, although the patients do not show increased basic metabolic rates[1,2]. This led us to conclude that our procedure of isolating mitochondria causes selection of loosely coupled mitochondria from a mixture of loosely and tightly coupled mitochondria, present in diseased muscle. The subsarcolemmal space of the affected muscle fibres shows the most pronounced morphological changes[1,2]. We concluded therefore that mild fragmentation of the muscle preferentially releases subsarcolemmal mitochondria.

The present paper shows that skeletal muscle from non-diseased white rats, on mild fragmentation, also preferentially releases mitochondria with a more loosely coupled state of oxidative phosphorylation when compared to the mitochondria released after more thorough homogenization of the muscle.

Mitochondria were isolated from 3 g of masseter muscle of rats. The muscles were chopped in two perpendicular directions with a McIlwain tissue slicer (0.1 mm distance between the cuts). The mince was then evenly suspended, by cutting with a pair of scissors, in 30 ml of isolation medium (pH 7.4), containing: 50 mM Tris–HCl, 100 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 1 mM EDTA and 0.5 mg of bovine serum albumin per ml. The mince was stirred for 5 min and part of the mince homogenized

TABLE I

OXIDATIVE PHOSPHORYLATION OF SKELETAL MUSCLE MITOCHONDRIA

The isolation of the mitochondria is described in the text. Oxygen uptake was measured with differential manometers. The reaction medium contained 25 mM glucose, 0.03 mM cytochrome *c*, 0.1 mM L-malate, 2.5 mM $MgCl_2$, 20 mM potassium phosphate buffer, 0.5 mM ATP, 0.5 mM EDTA, 50 mM KCl, 25 mM Tris–HCl buffer, 0.75 mg bovine serum albumin, about 1 mg of mitochondrial protein, and 15 mM glutamate (Expt. 1) or 15 mM pyruvate (Expt. 2). The centre well of the manometer vessels was provided with KOH and a filter paper. The side arm of the flasks contained 1.4 units (μmoles/min) of hexokinase (EC 2.7.1.1). The reaction volume was 1 ml, the temperature 25° and the pH 7.5. Readings were taken at regular intervals. After 20–24 min hexokinase was added from the side arm and after 20 min the reaction was stopped by the addition of perchloric acid (final concn. 4 %). The measurement of oxygen uptake and of phosphorylation, the calculation of the P/O ratio and the respiratory control index (RCI) were carried out as described before[1], except that phosphoglucose isomerase (EC 5.3.1.9) was included in the glucose-6-phosphate assay. Acid phosphatase activity was determined as described in ref. 3.

Expt. No.	*Type of homogenizer used*	Q_{O_2} (+ *hexokinase*)	*RCI*	*P/O*	*Yield of heavy mitochondria (mg protein/g muscle, wet wt.)*	*Relative acid phosphatase activity**
1	Loose	65	3.3	2.5	1.6	0.37
	Tight	87	5.4	2.6	2.5	0.24
2	Loose	98	2.6	2.6	3.4	0.20
	Tight	135	4.5	2.7	6.7	0.11

* For definition and unit see text.

in a loose-fitting homogenizer (5 strokes in a Potter–Elvehjem type homogenizer with a 'Teflon' pestle and a clearance of 0.25 mm (Expt. 1) or 3 strokes in an all glass homogenizer with a clearance of 0.15 mm (Expt. 2) and another part of the mince homogenized (5 strokes in Expt. 1; 20 strokes in Expt. 2) in a tight 'Teflon'-glass homogenizer (clearance, 0.10 mm). Particles sedimenting between 600 × *g* (3 min) and 4500 × *g* (10 min) were isolated by differential centrifugation and suspended in the complete isolation medium. All operations were carried out at 0–4°.

It can be seen from the experiments shown in Table I that the use of a tighter homogenizer not only improves the yield of the isolated muscle mitochondria, but also their quality, when a high respiratory control index may be used as a criterion for the intactness of the function of mitochondria. The release of the small amount of mitochondria by the use of the loose-fitting homogenizer was accompanied by the release of practically all of the β-glucuronidase (EC 3.2.1.31) and acid phosphatase (EC 3.1.3.2) activities from the minced muscle. The latter is reflected (Table I) by the relative acid phosphatase activity, defined as the total activity (in units) in the 600 × *g* supernatant divided by the amount of mitochondrial protein (in mg) isolated from the 600 × *g* supernatant. Since lysosomes are most concentrated in the perinuclear (subsarcolemmal) region, these findings indicate that mild fragmentation of muscle predominantly releases subsarcolemmal mitochondria.

Whether these mitochondria have a more loosely coupled oxidative phosphorylation due to aging[4], a process to which lysosomal activity could contribute[5], or whether the tightness of the coupling of oxidative phosphorylation is another difference between the various types of skeletal muscle mitochondria which can be distinguished (*cf.* ref. 6), still remains to be investigated.

Finally it may be of interest to note that SCHMALBRUCH[7] recently published that fibers of human larynx muscles show lateral dilatations of the sarcolemma, containing loosely disposed nuclei, lysosomes, lipofuscingranules and mitochondria, often with cristae arranged concentrically.

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